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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	H. Zaghouani	Group Art Unit 2644
Appl. No.	:	08/779,767	I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on
Filed	:	January 7, 1997	(Date)
For	:	COMPOUNDS, COMPOSITIONS AND METHODS FOR THE ENDOCYTIC PRESENTATION OF IMMUNOSUPPRESSIVE FACTORS	Daniel Hart, Reg. No. 40,637
Examiner	:	P. Nolan	

DECLARATION UNDER 37 C.F.R §1.132

Assistant Commissioner for Patents

Washington, D.C. 20231

Dear Sir:

1. This Declaration is being submitted to demonstrate that compositions comprising T cell receptor antagonists derived from proteins other than PLP are able to prevent T cell activation *in vivo*.

2. I am an inventor of the above-identified application and I am familiar with the specification and prosecution history.

3. As was demonstrated previously in the Declaration submitted on October 18, 2000, antagonist chimeras derived from myelin basic protein (MBP) were effective in suppressing EAE induced by MBP3 free peptide. Briefly, seven week old mice were induced for EAE by subcutaneous injection. When signs of paralysis became apparent, the mice were injected with Ig-MBP3A (an immunoglobulin comprising a T cell receptor antagonist derived from myelin basic protein) or IgW control antibody (which does not have any MBP sequences) at

11, 16, and 21 days post-induction. As can be seen in Exhibit E of the declaration submitted on October 18, 2000, the treatment of mice with the antagonist chimera Ig-MBP3A prevented the disease from taking a normal course, and mice did not have symptoms exceeding a mild loss of tail tone for the entire 50 day clinical assessment period. Therefore, the MBP-derived antagonist immunoglobulin chimeras were able to supress EAE over a long period.

4. The following experiments demonstrate that immunoglobulins containing T cell receptor antagonists derived from myelin basic protein prevent T cell activation *in vivo*.

In a control expirement, mice were each immunized with 50 µg Ig-MBP3 (an Ig chimera carrying an immunogenic amino acid sequence comprising amino acids 87-99 of myelin basic protein) and 150 µg Ig-W in 200 µl PBS CFA (1v 1v). After 10 days, the mice were sacrificed and spleen cells were isolated. The spleen cells were stimulated with whole MPB (0.5 µM) or the negative control HA peptide (15 µM). Thymidine incorporation was measured after 3 days of incubation. Each bar represents the mean + SD of triplicates after deduction of background cpms obtained with no stimulator in the medium. As illustrated in Panel A of Exhibit A, mice immunized with Ig-MBP3 and Ig-W developed a significant response against MBP. The response is specific for MBP because the isolated spleen cells showed no proliferation against the negative control HA peptide.

In a separate expirement, mice were each immunized with 50 µg Ig-MBP3 and 150 µg Ig-MBP3A (an Ig chimera comprising a T cell receptor antagonist derived from MBP) in 200 µl PBS/CFA (1v/1v). After 10 days, their splenic cells were harvested and stimulated with whole MPB (0.5 µM) or the negative control HA peptide (15 µm). Thymidine incorporation was measured after 3 days of incubation. Each bar represents the mean + SD of triplicates after deduction of background cpms obtained with no stimulator in the medium. In contrast to the results of the above control experiment, as illustrated in Panel B of Exhibit A, isolated spleen cells from mice that were immunized with a mixture of Ig-MBP3 and Ig-MBP3A showed a complete inhibition of activation (similar to that of the negative control HA peptide). Therefore, the immunoglobulin comprising a T cell receptor antagonist derived from MBP protein prevented T cell activation *in vivo*.

5. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: _____ By: _____

Habib Zaghouani

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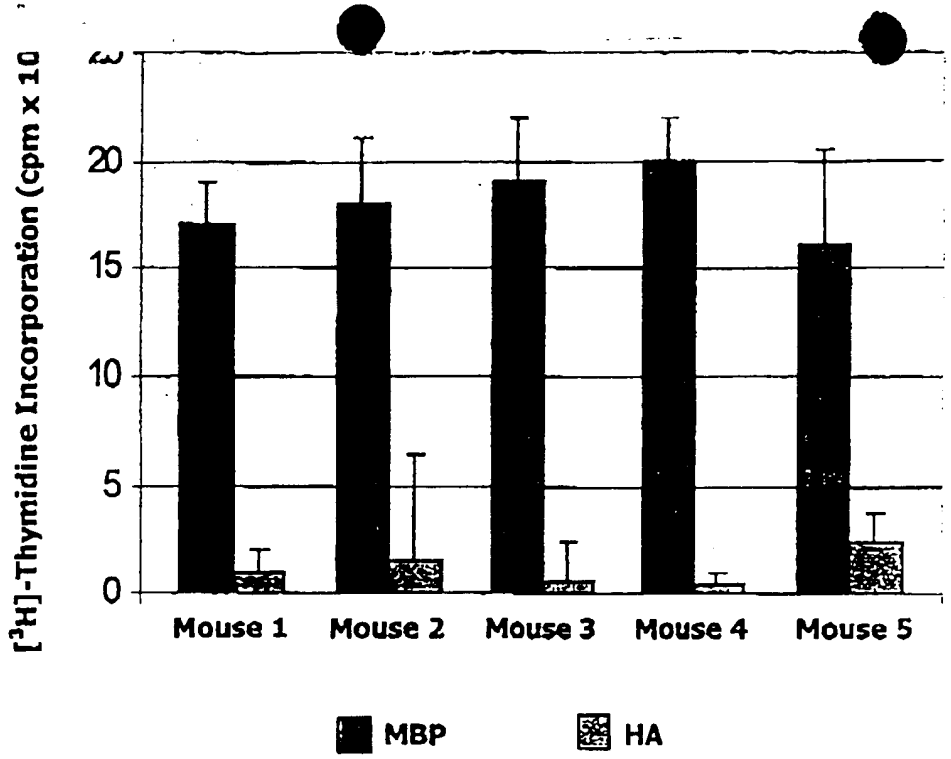
[³H]-Thymidine Incorporation (cpm x 10
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15
10
5
0
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
MBP
HA

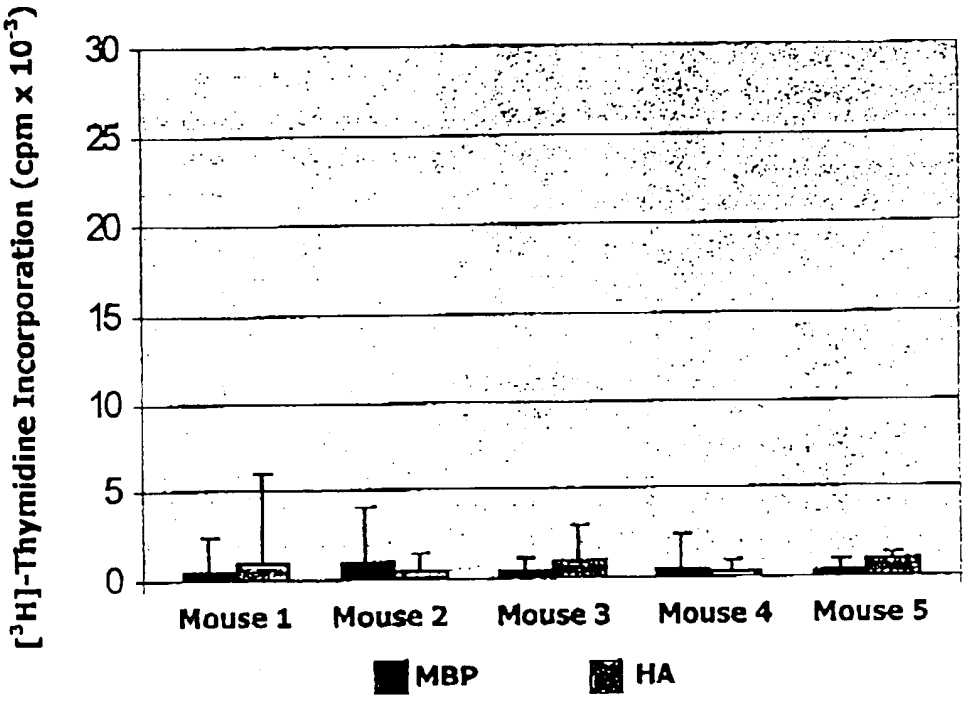
[³H]-Thymidine Incorporation (cpm x 10⁻³)
30
25
20
15
10
5
0
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
MBP
HA

EXHIBIT A